

***Astrothelium subsiamense* sp. nov. from Fujian, China**

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ABSTRACT—A new species, *Astrothelium subsiamense* from China, is described based on morphological, chemical, and molecular analyses. The new lichen is most similar to *A. siamense* but differs in its 3-septate and smaller ascospores. Its relationship with other *Astrothelium* spp. is presented, based on molecular phylogeny, and a key to the *Astrothelium* species recorded in China is also provided.

KEY WORDS—lichenized fungi, taxonomy, South China, *Trypetheliaceae*, *Trypetheliales*

Introduction

Astrothelium Eschw. (*Trypetheliaceae*, *Trypetheliales*, *Dothideomycetes*) was established by Eschweiler (1824) with *A. conicum* as the type species.

Astrothelium, the largest genus in *Trypetheliaceae*, comprises about 250 species worldwide (Aptroot & Lücking 2016, Wijayawardene & al. 2017, Jiang & al. 2021). The genus was originally limited to species in *Trypetheliaceae* with lateral fused ostioles and transversely septate ascospores (Aptroot & Lücking 2016, Luangsuphabool & al. 2016), but some workers (Harris 1995, Aptroot & al. 2008) considered this circumscription to be vague. Nelsen & al. (2014) have demonstrated that there is a strong conflict between molecular-based phylogeny and traditional morphological classification within *Trypetheliaceae*. With a recent generic rearrangement, *Astrothelium* now includes most species previously recognized as the artificial genera *Astrothelium*, *Campylothelium* Müll. Arg., *Cryptothelium* A. Massal., *Laurera* Rchb., and *Trypethelium* Spreng. (Aptroot & Lücking 2016; Lücking & al. 2016 a,b).

Astrothelium is characterized by a corticate thallus that is mostly olive-green (sometimes pale yellow) with lichexanthone (Hyde & al. 2013), simple to aggregate or pseudostromatic ascomata with apical to lateral and separate or fused ostioles, and ascomata or pseudostromata (either immersed or prominent) with pseudostromata often differing in structure and colour from the thallus. The ascospores in *Astrothelium* are often hyaline, transversely septate with diamond-shape lumina or muriform (Aptroot & Lücking 2016), although *A. fuscoporum* Soto-Medina & al. is known to produce pigmented ascospores (Soto-Medina & al. 2017).

Seven *Astrothelium* species have been previously reported from China: *A. cinnamomeum* (Eschw.) Müll. Arg., *A. variolosum* (Ach.) Müll. Arg., *A. sinense* S.H. Jiang & C. Zhang, *A. macrocarpum* (Fée) Aptroot & Lücking, *A. leucosessile* Lücking & al., *A. siamense* Luangsaph & al., and *A. subinterjectum* Lücking & al. (Aptroot & Seaward 1999; Jiang & al. 2021; Zahlbruckner 1932; Zhang 2020). A report of “*A. speciosum*” from China (Zahlbruckner 1933) is a typographic error for *Anthracotheceium speciosum* Zahlbr. (Aptroot & Lücking 2016). Here we report a new species, *A. subsiamense*, as an additional record from Fujian province, South China.

Materials & methods

Specimens, morphology, chemistry

The specimens of the new species were collected from Fujian province of China and deposited in the Fungarium of College of Life Sciences, Liaocheng University, Liaocheng, China (LCUF). The morphology and anatomy were observed and photographed using a Olympus SZX16 dissecting microscope and Olympus BX53 compound microscope. Amyloidity of the ascospores was tested using Lugol's solution. Secondary metabolites were examined by colour test (10% KOH, saturated solution NaClO, and p-phenylenediamine dissolved in ethanol) and thin-layer chromatography (TLC) using solvent C (Culberson 1972, Culberson & Kristinsson 1970).

DNA extraction, PCR sequencing, phylogenetic analysis

Genomic DNA was extracted from ascomata of the specimens using Sigma-Aldrich REDExtract-N-AmpTM Plant PCR Kit according to the manufacturer's protocol and amplified using the ITS1F and ITS4 primer pair (White & al. 1990). The 50 µL PCR reaction system consisted of 2 µL each primer solution, 2 µL genomic DNA, 19 µL ddH₂O, and 25 µL 2×Taq PCR MasterMix. The PCR protocol comprised an initial denaturation for 3 min at 94°C, 35 cycles at 94°C for 30 s + 52°C for 30 s + extension at 72°C for 90 s, and a final extension at 72°C for 10 min. The PCR amplicon was affirmed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai, China). Since the specimens in this study were slightly old, the genomic DNA was successfully extracted from only one specimen. The newly generated

TABLE 1. Specimen and sequences used in the phylogenetic analysis

SPECIES	SPECIMEN	GENBANK (ITS)
<i>Astrothelium aenascens</i>	HRK93	LC127385
	HRK98	LC127386
<i>A. flavocoronatum</i>	TSL63	AB758900
	KY859	LC127381
<i>A. macrostiolum</i>	PHL84	LC127389
<i>A. neglectum</i>	TAK8	LC127392
	TAK12	LC127393
	TAK17	LC127394
<i>A. neovariolosum</i>	KY777	LC127390
	KY848	LC127391
<i>A. siamense</i>	KRB105	LC127387
	KRB139	LC127388
<i>A. sinense</i>	GD19282	MT948055
	GD19156	MT948056
<i>A. subsiamense</i>	FJ19151	OM001640
<i>Trypethelium eluteriae</i>	GD19106	MT420802
	GD19109	MT420803

Note: The new sequence obtained in this study is shown in bold font.

sequence was submitted to GenBank. Sixteen related ITS sequences for phylogenetic tree construction were downloaded from GenBank, and *Trypethelium eluteriae* Spreng. was selected as the outgroup (Jiang & al. 2021) (TABLE1).

Contigs were assembled and edited using the program Seqman. The ClustalW Multiple alignment and edit were undertaken by Bioedit v. 7.0.5, which yielded final alignment of 652 bp. Maximum likelihood (ML) was performed using the CIPRES Scientific gateway portal (<http://www.phylo.org/portal2/>; Miller & al. 2010). Maximum likelihood bootstrapping analysis was performed with RAxML-HPIC BlackBox v. 8.2.12 (Stamatakis 2014), using the default parameters as implemented on the CIPRES, NSF XSEDE resource with bootstrap statistics calculated from 1000 bootstrap replicates. Generated phylogenetic tree was visualized and edited under Figtree v1.4.2. In addition, Neighbor-Joining analysis was operated on MEGA7 involving 1000 replicates (Kumar & al. 2016).

Phylogenetic results

The maximum likelihood (ML) and Neighbor-Joining analysis (NJ) phylogenetic trees exhibited the same topology; we therefore present only the

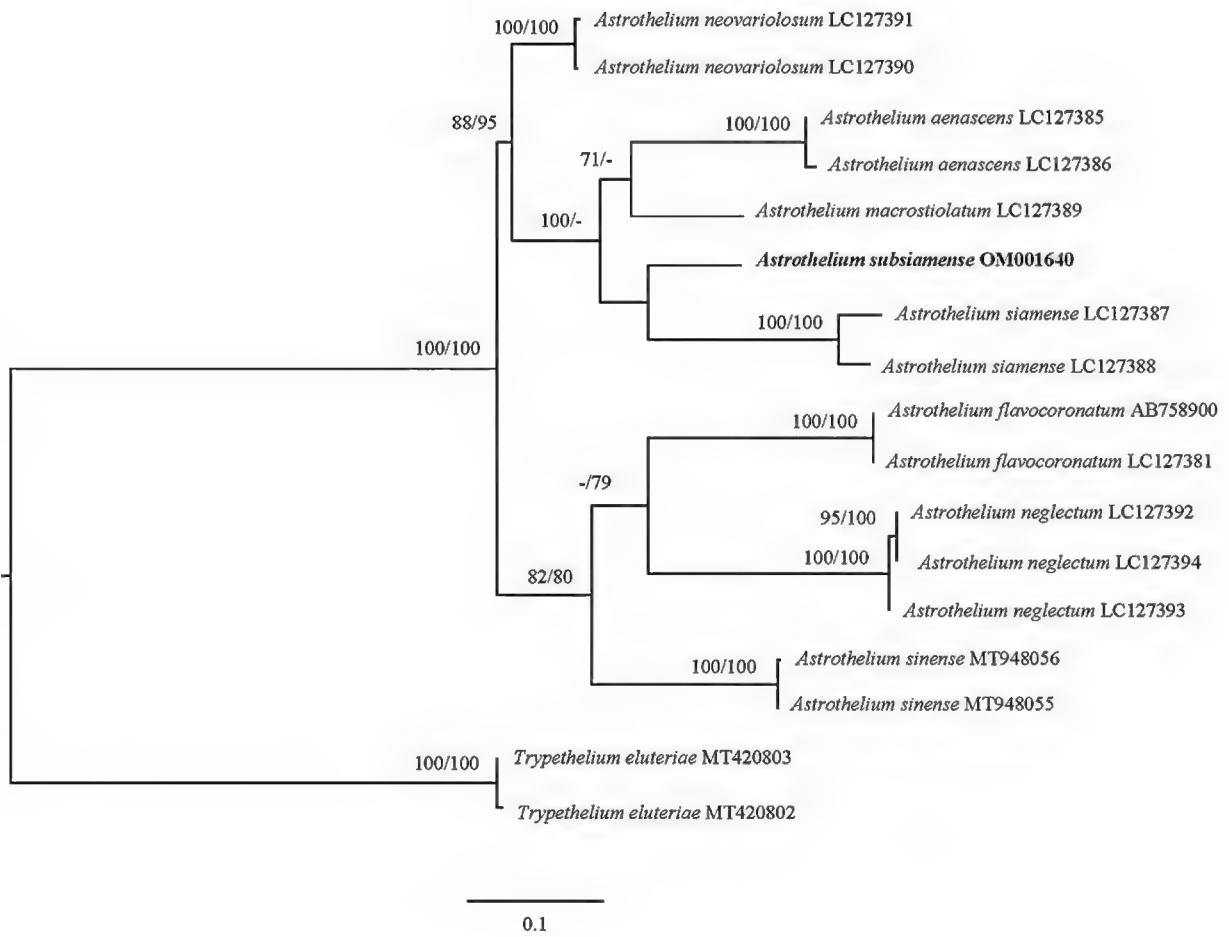


FIG. 1. Phylogenetic tree of *Astrothelium* based on ITS. ML and NJ bootstrap support >70% are shown at the nodes (ML/NJ). The new sequence is set in bold font. Scale bar = 0.1 substitution.

ML tree (FIG. 1), based on 17 ITS sequences from 9 taxa. Both trees supported *Astrothelium subsiamense* as well separated from other *Astrothelium* species and closely related to *A. siamense*.

Taxonomy

Astrothelium subsiamense Y.F. Zhao & Z.F. Jia, sp. nov.

FIG. 2

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Differs from *Astrothelium siamense* by its 3-septate and smaller ascospores.

TYPE: China. Fujian Province: Quanzhou City, Mt. Jiuxianshan, Natural Observation Path, 25.7122°N 118.1214°E, alt. 1460 m, on bark, 25 Jul. 2019, F.Y. Liu (Holotype, LCUF FJ19151; GenBank OM001640).

ETYMOLOGY: from *sub-* and *siamense*, referring to the similarity with *Astrothelium siamense*.

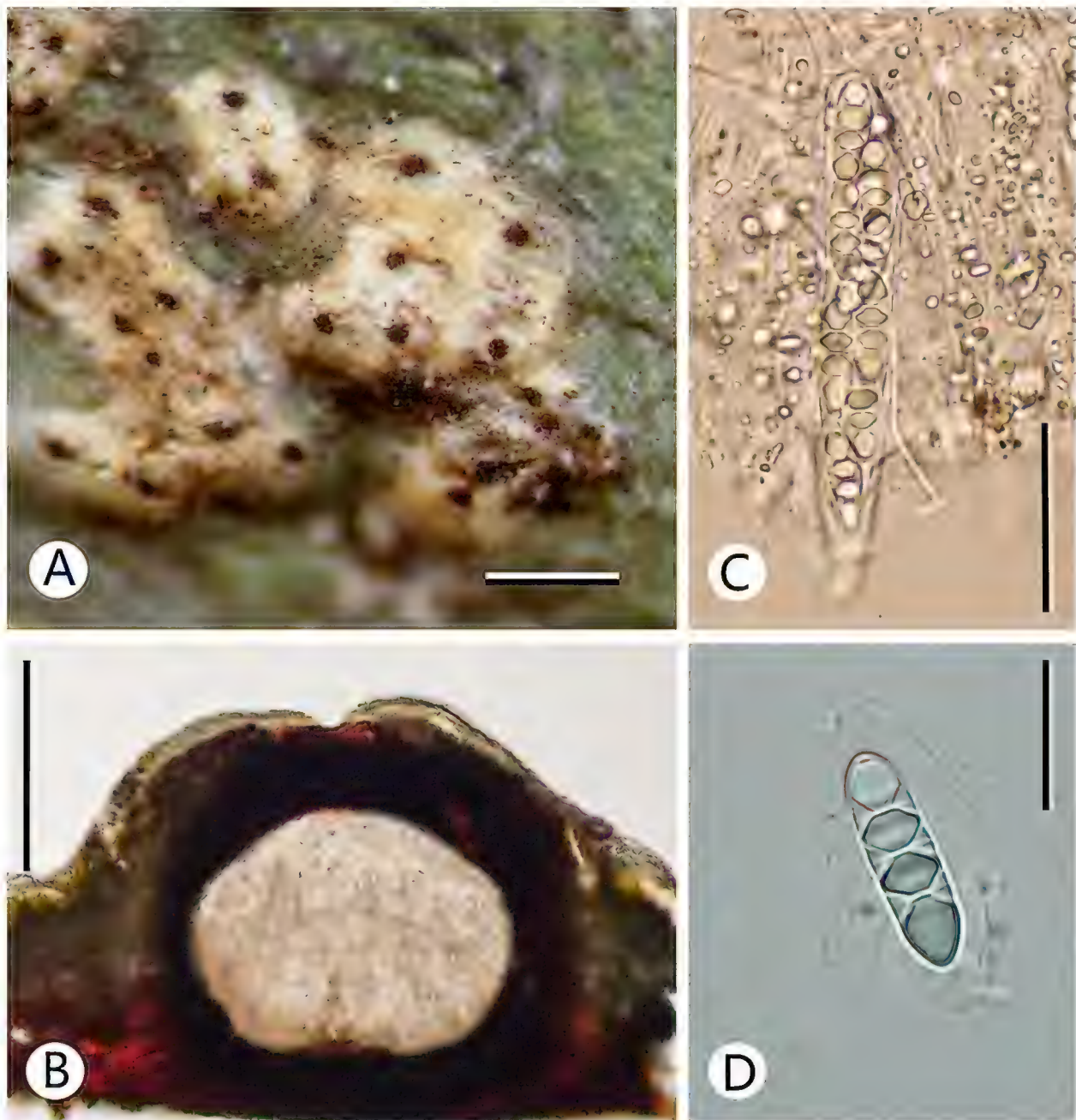


FIG. 2. *Astrothelium subsiamense* (holotype, FJ19151): A. Thallus with ascomata; B. Perithecial section; C. Ascus; D. Ascospores. Scale bars: A = 0.5 mm; B = 200 μ m; C = 50 μ m; D = 20 μ m.

THALLUS crustose, corticate, continuous, olive-green to greyish-green, smooth, shiny, 20–40 μ m thick; PHOTOSYNTHETIC symbiotic alga *Trentepohlia*; ASCOMATA perithecia, pyriform, 0.2–0.5 mm diam, mostly aggregated groups immersed in pseudostromata; PSEUDOSTROMATA pale yellow to white, 0.7–3.5 mm wide; OSTIOLES apical, black, flat to sunk; ASCOMATAL WALL carbonized, ≤ 100 μ m thick; HAMATHECIUM inspersed with oil droplets; ASCI hyaline, clavate, $100\text{--}114 \times 15\text{--}20$ μ m, 8-spored, biseriate; ASCOSPORES fusiform, hyaline, 3-septate, with diamond-shape lumina, $20\text{--}32 \times 7\text{--}10$ μ m, surrounded by gelatinous sheath, 2–9 μ m. Pycnidia not seen.

CHEMISTRY—Thallus K+ yellow green, C–, KC–, P–, UV+ yellow. Pseudostromata K+ yellow green, C–, KC–, P–, UV–. TLC: lichenxanthone (thallus).

ECOLOGY & DISTRIBUTION—On bark of subtropic forest park; known only from the type locality in Fujian.

ADDITIONAL SPECIMEN EXAMINED: CHINA, FUJIAN, Quanzhou City, Mt. Jiuxianshan, Natural Observation Path, 25.7122°N 118.1214°E, 25 Jul. 2019, F. Y. Liu (LCUF FJ19157).

Discussion

Astrothelium subsiamense is characterized by its olive-green to greyish-green thallus, mostly aggregated ascomata immersed in pseudostromata, hamathecium inspersed with droplets, and lichenxanthone found only in the thallus. *Astrothelium siamense* and *A. nitidiusculum* (Nyl.) Aptroot & Lücking are morphologically similar to the new species; *A. siamense* differs in its larger ascospores (4–7-septate, 30–50 × 10.5–12.0 µm; (Luangsuphabool & al. 2016), while *A. nitidiusculum* is distinguished by its clear hamathecium without droplets and absence of secondary metabolites (Aptroot & Lücking 2016). Our new species also resembles *A. grossoides* Aptroot & Lücking in having 3-septate ascospores and inspersed hamathecium, but *A. grossoides* has an uneven-bullate thallus and ascomata often containing lichexanthone (Aptroot & Lücking 2016). *Astrothelium subsiamense* shares a similar thallus and ascospores with *A. sinense*, but *A. sinense* has a thicker carbonized ascomatal wall and contains lichenxanthone in its pseudostromata (Jiang & al. 2021).

Key to the species of *Astrothelium* known from China

- 1. Ascospores 4–7-septate *A. siamense*
- 1. Ascospores 3-septate.....2
- 2. Hamathecium clear.....3
- 2. Hamathecium inspersed with oil droplets.....6
- 3. Pseudostromata with yellow to orange pigment.....4
- 3. Pseudostromata without pigment.....5
- 4. Thallus UV–*A. cinnamomeum*
- 4. Thallus UV+, yellow*A. macrocarpum*
- 5. Thallus UV–*A. subinterjectum*
- 5. Thallus UV+, yellow *A. variolosum*
- 6. Ascomata forming pseudostromata7
- 6. Ascomata simple or aggregated..... *A. leucosessile*
- 7. Pseudostromata UV– *A. subsiamense*
- 7. Pseudostromata UV+, yellow*A. sinense*

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